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(54) **DETECTION OF WHEAT AND BARLEY
 FUNGAL PATHOGENS WHICH ARE
 RESISTANT TO CERTAIN FUNGICIDES
 USING THE POLYMERASE CHAIN
 REACTION**

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 See application file for complete search history.

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(57) **ABSTRACT**

Primers specific for races of pathogenic fungi which are
 resistant to certain fungicides are used in polymerase chain
 reaction assays for the detection of fungal pathogens. The
 use of these primers enables the detection of specific isolates
 of fungal pathogens and the monitoring of disease develop-
 ment in plant populations. The invention includes DNA
 sequences which show variability between different fungal
 pathotypes. Such DNA sequences are useful in the method
 of the invention as they can be used to derive primers for use
 in polymerase chain reaction (PCR)-based diagnostic
 assays. These primers generate unique fragments in PCR
 reactions in which the DNA template is provided by specific
 fungal pathotypes and can thus be used to identify the
 presence or absence of specific pathotypes in host plant
 material before the onset of disease symptoms.

8 Claims, No Drawings

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DETECTION OF WHEAT AND BARLEY FUNGAL PATHOGENS WHICH ARE RESISTANT TO CERTAIN FUNGICIDES USING THE POLYMERASE CHAIN REACTION

This application claims the benefit of U.S. Provisional Patent Application Ser. No. 60/369,796 filed Apr. 3, 2002, which is incorporated by reference in its entirety.

FIELD OF THE INVENTION

The present invention relates to the use of primers specific for races of pathogenic fungi which are resistant to certain fungicides in polymerase chain reaction assays for the detection of fungal pathogens. The use of these primers enables the detection of specific isolates of fungal pathogens and the monitoring of disease development in plant populations.

BACKGROUND OF THE INVENTION

Diseases in plants cause considerable crop loss from year to year resulting both in economic deprivation to farmers and additionally in many parts of the world to shortfalls in the nutritional provision for local populations. The widespread use of fungicides has provided considerable security against plant pathogen attack. However, despite \$1 billion worth of expenditure on fungicides, worldwide crop losses amounted to approximately 10% of crop value in 1981 (James, 1981; *Seed Sci. & Technol.* 9: 679-685).

The severity of the destructive process of disease depends on the aggressiveness of the pathogen and the response of the host. One aim of most plant breeding programs is to increase the resistance of host plants to disease. Typically, different races of pathogens interact with different varieties of the same crop species differentially, and many sources of host resistance only protect against specific pathogen races. Furthermore, some pathogen races show early signs of disease symptoms, but cause little damage to the crop. Jones and Clifford (1983; *Cereal Diseases*, John Wiley) report that virulent forms of the pathogen are expected to emerge in the pathogen population in response to the introduction of resistance into host cultivars and that it is therefore necessary to monitor pathogen populations. In addition, there are several documented cases of the evolution of fungal strains which are resistant to particular fungicides. As early as 1981, Fletcher and Wolfe (1981; *Proc. 1981 Brit. Crop Prot. Conf.*) contended that 24% of the powdery mildew populations from spring barley, and 53% from winter barley showed considerable variation in response to the fungicide triadimenol and that the distribution of these populations varied between varieties with the most susceptible variety also giving the highest incidence of less susceptible types. Similar variation in the sensitivity of fungi to fungicides has been documented for wheat mildew (also to triadimenol), *Botrytis* (to benomyl), *Pyrenophora* (to organomercury), *Tapesia* (to MBC-type fungicides) and *Mycosphaerella fijiensis* to triazoles to mention just a few (Jones and Clifford; *Cereal Diseases*, John Wiley, 1983).

Cereal species are grown world-wide and represent a major fraction of world food production. Although yield loss is caused by many pathogens, the necrotizing pathogens *Septoria* and *Tapesia* are particularly important in the major cereal growing areas of Europe and North America (Jones and Clifford; *Cereal Diseases*, John Wiley, 1983). In particular, the differential symptomatology caused by different

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isolates and species of these fungi make the accurate predictive determination of potential disease loss difficult. Consequently, the availability of improved diagnostic techniques for the rapid and accurate identification of specific pathogens will be of considerable use to field pathologists.

The eyespot disease of cereals is caused by the fungi *Tapesia yallundae* and *Tapesia acuformis* is restricted to the basal culm of the plant. The two causal pathogens were previously classified as two subspecies of *Pseudocercospora herpotrichoides* (Fron) Deighton (anamorph). *T. yallundae* refers to the variety herpotrichoides and the SF-, L-, I- or W-types. *T. acuformis* corresponds to the variety acuformis and the FE-, N-, II- or R-types (Leroux and Gredt, 1997; 51:321-327). Wheat, rye, oats and other grasses are susceptible to the eyespot disease which occurs in cool, moist climates and is prevalent in Europe, North and South America, Africa and Australia. Wheat is the most susceptible cereal species, but isolates have been identified which are also virulent on other cereals. The R-strain (*T. acuformis*) of the fungus, for example, has also been isolated from rye and grows more slowly on wheat than the W-strain (*T. yallundae*) which has been isolated from wheat. Although eyespot may kill tillers or plants outright, it more usually causes lodging and/or results in a reduction in kernel size and number. Yield losses associated with eyespot are of even greater magnitude than those associated with *Septoria tritici* and *Septoria nodorum*. Typical control measures for eyespot include treatment with growth regulators to strengthen internodes, and fungicide treatment. However, the differing susceptibility of cultivars to different strains of the fungus render the predictive efficacy of fungicide treatments difficult. In addition, both Leroux et al (1997; *Pesticide Science*, 51:321-327) and Dyer et al (2000; *Appl. and Environ. Microbiol.* 66:4599-4604) have reported on isolates of *T. yallundae* with reduced sensitivity to the imidazole DMI fungicide prochloraz (1-[N-propyl-N-[2-92,4,6-trichlorophenoxy]ethyl]carbonyl]-imidazole). Following heavy treatments of benzimidazole fungicides such as benomyl, carbendazim and thiabendazole, acquired resistance to this class of fungicides was determined in both *T. acuformis* and *T. yallundae* (Leroux and Cavelier, 1983; *Phytoma* 351:40) and (Cavelier et al, 1985; *Bull. OEPP* 85:495).

Thus, there is a real need for the development of technology which will allow the identification of specific races of pathogen fungi which are resistance to certain fungicides early in the infection process. By identifying the specific race of a pathogen before disease symptoms become evident in the crop stand, the agriculturist can assess the likely effects of further development of the pathogen in the crop variety in which it has been identified and can choose an appropriate fungicide if such application is deemed necessary.

SUMMARY OF THE INVENTION

The present invention relates to the use of primers specific for races of pathogenic fungi which are resistant to certain fungicides in polymerase chain reaction assays for the detection of fungal pathogens. The invention provides DNA sequences which show variability between different fungal pathotypes. Such DNA sequences are useful in the method of the invention as they can be used to derive primers for use in polymerase chain reaction (PCR)-based diagnostic assays. These primers generate unique fragments in PCR reactions in which the DNA template is provided by specific fungal pathotypes and can thus be used to identify the

presence or absence of specific pathotypes in host plant material before the onset of disease symptoms.

This invention provides the possibility of assessing potential damage in a specific crop variety-pathogen strain relationship and of utilizing judiciously the diverse armory of fungicides which is available. Furthermore, it can be used to provide detailed information on the development and spread of specific pathogen races over extended geographical areas.

Kits useful in the practice of the invention are also provided. The kits find particular use in the identification of *Tapesia* pathogens.

The present invention provides a nucleic acid molecule comprising the nucleic acid sequence of SEQ ID NOS: 3-13 or 14. In a more preferred embodiment, the nucleic acid molecule has sequence identity with at least 10 contiguous nucleotides of SEQ ID NOS: 2-13 or 14. In another preferred embodiment, the nucleic acid molecule comprises a nucleotide sequence of SEQ ID NOS: 3-76 or 77.

The invention also provides a pair of oligonucleotide primers wherein at least one primer consists of the nucleotide sequence of SEQ ID NOS: 3-76 or 77. In a preferred embodiment, the pair of oligonucleotide primers comprises:

JB944 (SEQ ID NO:59) and JB943 (SEQ ID NO:58);
JB944 (SEQ ID NO:59) and JB945 (SEQ ID NO:60);
JB934 (SEQ ID NO:49) and JB935 (SEQ ID NO:50); and
JB937 (SEQ ID NO:52) and JB935 (SEQ ID NO:50).

The invention also provides a method for the detection of a fungal pathogen, comprising the steps of:

- (a) isolating DNA from a plant tissue infected with a pathogen;
- (b) subjecting said DNA to polymerase chain reaction amplification using at least one primer having sequence identity with at least 10 contiguous nucleotides of a randomly amplified polymorphic DNA sequence of a *Tapesia* spp.; and
- (c) detecting said fungal pathogen by visualizing the product or products of said polymerase chain reaction amplification.

In a preferred embodiment, the fungal pathogen is *Tapesia yallundae*, *Tapesia acuformis*. More preferably, the *Tapesia yallundae* is subtype Ic., and *Tapesia acuformis* subtypes IIs or IIp. In another preferred embodiment, at least one primer having the nucleotide sequence of SEQ ID NOS: 3-76 or 77.

The invention further provides a method for the detection of a fungal pathogen, comprising the steps of:

- (a) isolating DNA from a plant tissue infected with a pathogen;
- (b) subjecting said DNA to polymerase chain reaction amplification using at least one primer having sequence identity with at least 10 contiguous nucleotides of a randomly amplified polymorphic DNA from a *Tapesia* spp.; and
- (c) detecting said fungal pathogen by visualizing the product or products of said polymerase chain reaction amplification.

In a preferred embodiment, the fungal pathogen is *Tapesia yallundae*, *Tapesia acuformis*. More preferably, the *Tapesia yallundae* is subtype Ic, and *Tapesia acuformis* subtypes IIs or IIp. In another preferred embodiment, at least one primer having the nucleotide sequence of SEQ ID NOS: 3-76 or 77. In more preferred embodiment, the pair of primers comprises:

JB944 (SEQ ID NO:59) and JB943 (SEQ ID NO:58);
JB944 (SEQ ID NO:59) and JB945 (SEQ ID NO:60);
JB934 (SEQ ID NO:49) and JB935 (SEQ ID NO:50); or
JB937 (SEQ ID NO:52) and JB935 (SEQ ID NO:50).

The invention also provides a diagnostic kit used in detecting a fungal pathogen comprising at least one primer having at least 10 contiguous nucleotides of a nucleic acid molecule of the nucleic acid molecules described above. In a preferred embodiment, at least one primer comprises SEQ ID NO: 3-76 or 77. In more preferred embodiments, the pair of primers are:

JB944 (SEQ ID NO:59) and JB943 (SEQ ID NO:58);
JB944 (SEQ ID NO:59) and JB945 (SEQ ID NO:60);
JB934 (SEQ ID NO:49) and JB935 (SEQ ID NO:50); or
JB937 (SEQ ID NO:52) and JB935 (SEQ ID NO:50).

Brief Description of the Sequences in the Sequence Listing

SEQ-ID-NO: 1	M13 Sequencing Forward Primer
SEQ-ID-NO: 2	M13 Sequencing Reverse Primer
SEQ-ID-NO: 3	RAPD-PCR Clone Ib1-27
SEQ-ID-NO: 4	RAPD-PCR Clone Ib2-31
SEQ-ID-NO: 5	RAPD-PCR Clone Ib3-33
SEQ-ID-NO: 6	RAPD-PCR Clone Ic1-22
SEQ-ID-NO: 7	RAPD-PCR Clone Ic 020502Ic4and6
SEQ-ID-NO: 8	RAPD-PCR Clone Ic 020602D-20
SEQ-ID-NO: 9	RAPD-PCR Clone Ic 020602D-21
SEQ-ID-NO: 10	RAPD-PCR Clone IIp1-17
SEQ-ID-NO: 11	RAPD-PCR Clone IIp 020602A-11
SEQ-ID-NO: 12	RAPD-PCR Clone IIp 020602B-15
SEQ-ID-NO: 13	RAPD-PCR Clone IIp 020602B-16
SEQ-ID-NO: 14	RAPD-PCR Clone IIs2-39
SEQ-ID-NO: 15	JB900
SEQ-ID-NO: 16	JB901
SEQ-ID-NO: 17	JB902 Probe
SEQ-ID-NO: 18	JB903
SEQ-ID-NO: 19	JB904
SEQ-ID-NO: 20	JB905
SEQ-ID-NO: 21	JB906
SEQ-ID-NO: 22	JB907
SEQ-ID-NO: 23	JB908
SEQ-ID-NO: 24	JB909
SEQ-ID-NO: 25	JB910
SEQ-ID-NO: 26	JB911
SEQ-ID-NO: 27	JB912 Probe
SEQ-ID-NO: 28	JB913
SEQ-ID-NO: 29	JB914
SEQ-ID-NO: 30	JB915
SEQ-ID-NO: 31	JB916
SEQ-ID-NO: 32	JB917 Probe
SEQ-ID-NO: 33	JB918
SEQ-ID-NO: 34	JB919
SEQ-ID-NO: 35	JB920
SEQ-ID-NO: 36	JB921
SEQ-ID-NO: 37	JB922 Probe
SEQ-ID-NO: 38	JB923
SEQ-ID-NO: 39	JB924
SEQ-ID-NO: 40	JB925
SEQ-ID-NO: 41	JB926
SEQ-ID-NO: 42	JB927 Probe
SEQ-ID-NO: 43	JB928
SEQ-ID-NO: 44	JB929
SEQ-ID-NO: 45	JB930
SEQ-ID-NO: 46	JB931
SEQ-ID-NO: 47	JB932
SEQ-ID-NO: 48	JB933
SEQ-ID-NO: 49	JB934
SEQ-ID-NO: 50	JB935
SEQ-ID-NO: 51	JB936
SEQ-ID-NO: 52	JB937
SEQ-ID-NO: 53	JB938
SEQ-ID-NO: 54	JB939
SEQ-ID-NO: 55	JB940
SEQ-ID-NO: 56	JB941
SEQ-ID-NO: 57	JB942
SEQ-ID-NO: 58	JB943
SEQ-ID-NO: 59	JB944
SEQ-ID-NO: 60	JB945
SEQ-ID-NO: 61	JB946
SEQ-ID-NO: 62	JB947
SEQ-ID-NO: 63	JB948

-continued

Brief Description of the Sequences in the Sequence Listing	
SEQ-ID-NO: 64	JB949
SEQ-ID-NO: 65	JB950
SEQ-ID-NO: 66	JB951
SEQ-ID-NO: 67	JB952
SEQ-ID-NO: 68	JB953
SEQ-ID-NO: 69	JB954
SEQ-ID-NO: 70	JB955
SEQ-ID-NO: 71	JB956
SEQ-ID-NO: 72	JB957
SEQ-ID-NO: 73	JB958
SEQ-ID-NO: 74	JB959
SEQ-ID-NO: 75	JB960
SEQ-ID-NO: 76	JB961
SEQ-ID-NO: 77	JB962

DETAILED DESCRIPTION OF THE INVENTION

The present invention provides unique DNA sequences which are useful in identifying different pathotypes of plant pathogenic fungi. Particularly the DNA sequences can be used as primers in PCR based analysis for the identification of fungal pathotypes. The DNA sequences of the invention include products cloned from RAPD primer analysis of particular fungal pathogens as well as primers which are derived from these regions which are capable of identifying the particular pathogen. These DNA sequences from different pathotypes within a pathogen species or genus which vary between the different members of the species or genus based on different fungicides' susceptibility can be used to identify those specific members.

Biomedical researchers have used PCR-based techniques for some time and with moderate success to detect pathogens in infected animal tissues. Only recently, however, has this technique been applied to detect plant pathogens. The presence of *Gaumannomyces graminis* in infected wheat has been detected using PCR of sequences specific to the pathogen mitochondrial genome (Schlesser et al., 1991; *Applied and Environ. Microbiol.* 57: 553-556) and random amplified polymorphic DNA (i.e. RAPD) markers were able to distinguish numerous races of *Gremmeniella abietina*, the causal agent of scleroderris canker in conifers.

The DNA sequences of the invention are from randomly amplified polymorphic DNA (RAPD) of different plant pathogens. The RAPD sequences from different pathotypes within a pathogen species or genus vary between the different members of the species or genus. Once having determined the unique RAPD sequences of a pathogen, primers can be derived from the sequences. That is, primers can be designed based on regions within the uniquely identified RAPD fragment sequence among the fungal pathotypes. These sequences and primers based on these sequences can be used to identify specific pathogen members.

Particular DNA sequences of interest include uniquely identified RAPD sequences from *Tapesia*, particularly, *Tapesia acuformis* and *Tapesia yallundae*, more particularly for the identification of *T. acuformis* subtypes IIa and IIp and *T. yallundae* subtypes Ia, Ib and Ic. Such DNA sequences as well as primers of interest are given in SEQ ID NO: 3-77. The sequences find use in the PCR-based identification of the pathotypes of interest.

Sequences from RAPD analysis of uniquely identified fragments include SEQ-ID-NOs: 3-14. The sequences find use in the PCR-based identification of pathogens of interest.

In a preferred embodiment the sequence disclosed as SEQ-ID-NO: 10 is useful in the development of primers for differentiating *T. acuformis* subtypes IIa and IIp. In another preferred embodiment the sequence disclosed as SEQ-ID-NO: 8 is useful in the development of primers for the detection of *T. yallundae* subtype Ic.

Sequences from oligonucleotide primers derived from the uniquely identified RAPD analysis fragments are disclosed as SEQ-ID-Nos: 15-77. In a preferred embodiment, the pair of oligonucleotide primers consists of SEQ-ID-NO: 59 and SEQ-ID-NO: 58 is used for the detection of *T. yallundae* Ic. In another preferred embodiment, the pair of oligonucleotide primers consists of SEQ-ID-NO: 59 and SEQ-ID-NO: 60 is used for the detection of *T. yallundae* Ic. In yet other embodiments, *T. acuformis* subtype IIa can be differentiated from *T. acuformis* subtype IIp using the primer combination consisting of oligonucleotide primers with SEQ-ID-NO: 49 and SEQ-ID-NO: 50 and the primer combination consisting of oligonucleotide primers with SEQ-ID-NO: 52 and SEQ-ID-NO: 50.

The present invention lends itself readily to the preparation of "kits" containing the elements necessary to carry out the process. Such a kit may comprise a carrier being compartmentalized to receive in close confinement therein one or more container means, such as tubes or vials. One of said container means may contain unlabeled or detectably labeled DNA primers. The labeled DNA primers may be present in lyophilized form, or in an appropriate buffer as necessary. One or more container means may contain one or more enzymes or reagents to be utilized in PCR reactions. These enzymes may be present by themselves or in admixtures, in lyophilized form or in appropriate buffers.

Finally, the kit may contain all of the additional elements necessary to carry out the technique of the invention, such as buffers, extraction reagents, enzymes, pipettes, plates, nucleic acids, nucleoside triphosphates, filter paper, gel materials, transfer materials, autoradiography supplies, and the like.

The examples below show, without limitation, typical experimental protocols which can be used in the isolation of unique RAPD sequences, the selection of suitable primer sequences, the testing of primers for selective and diagnostic efficacy, and the use of such primers for disease and fungal isolate detection. Such examples are provided by way of illustration and not by way of limitation.

EXAMPLES

Standard recombinant DNA and molecular cloning techniques used here are well known in the art and are described by J. Sambrook, E. F. Fritsch and T. Maniatis, *Molecular Cloning: A Laboratory manual*, Cold Spring Harbor laboratory, Cold Spring Harbor, N.Y. (1989) and by T. J. Silhavy, M. L. Berman, and L. W. Enquist, *Experiments with Gene Fusions*, Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y. (1984) and by Ausubel, F. M. et al., *Current Protocols in Molecular Biology*, pub. by Greene Publishing Assoc. and Wiley-Interscience (1987).

Example 1

Fungal Isolates and Genomic Fungal DNA Extraction

See Table 1 for a listing of the fungal isolates used. Isolates for which fungicide sensitivity was characterized were obtained from Institut National de la Recherche

Agronomique (INRA, Le Rheu, France). Fungi are grown on PDA (Potato Dextrose Agar) plates. Cultures are incubated for up to 10 days at 28° C. Mycelia are ground in liquid nitrogen, and total genomic DNA is extracted using the following modified CTAB protocol.

1. Freeze-dried mycelium was homogenized in 1.5 ml Eppendorf tubes (two tungsten carbide 3 mm beads were added) using a Retsch mill (MM200, Retsch GmbH & Co., Haan, Germany).
2. Add 600 µl extraction buffer (700 mM NaCl, 50 mM Tris HCl, 10 mM EDTA, 1% β-mercapthoethanol, 1% CTAB) and incubate for 1 hr at 65° C., vortexing every 10–20 minute interval.
3. Add 400 µl chlorophorm:isoamylalcohol (24.1, v:v) shake for 15 min
4. Centrifuge for 10 min (16000 g)
5. Transfer the aqueous phase to a new tube and add 400 µl extraction buffer and 400 µl chloroform:isoamylalcohol
6. Shake for 15 min
7. Centrifuge 10 min
8. Transfer aqueous phase to a new tube
9. Add 0.6× volumes of isopropanol
10. Shake for 5 min
11. Centrifuge for 5 min
12. Discard supernatant and dry pellet
13. Wash pellet with cold EtOH 70%
14. Centrifuge 2 min
15. Dry pellet
16. Resuspend in 50 µl TE

Tris-HCl, pH8.3 and containing 100 µM of each dTTP, dATP, dCTP, and dGTP in 25 µL reactions. In each reaction, 25 pmol of RAPD primer is used with 0.5 Units of AmpliTaq Polymerase. Approximately 25 ng of genomic DNA from the subtypes listed in Example 1 are used as template. Reactions are run in a GeneAmp Model 9700 thermal cycler (Applied Biosystems, Foster City, Calif.). Thermal cycling is run for 45 cycles of 30 s at 94° C., 30 s at 34° C., and 60 s at 72° C. and is proceeded by a hold at 94° C. for one minute and followed by a final hold at 72° C. for ten minutes before being stored at 4° C. The products are analyzed by loading 10 µl of each PCR sample with loading buffer on a 1.0% agarose gel and electrophoresing.

The gel is stained with ethidium bromide and separated RAPD-PCR bands are observed under ultraviolet light.

Example 3

Selection, Cloning, and Sequencing of Subtype-Specific RAPD-PCR Products

RAPD-PCR products for each *Tapesia* spp. subtype are compared. Bands that appear to be specific to a certain subtype are selected for further analysis by DNA sequencing. These bands are cut from the agarose gel by a sterile scalpel. The RAPD-PCR product is purified from the agarose using GenElute Minus EtBr Spin Columns (Product Code 5-6501, Sigma-Aldrich, St. Louis, Mo., USA). The purified product is cloned into the pCR4-TOPO vector and

TABLE 1

Identification of Test Isolates						
Fungal	Fungal Subtype		Fungicide Sensitivity ¹			Cyprodinil
Species	Isolate	Identifier	Triadimenol	Prochloraz	Carbendazim	(Unix)
<i>Tapesia yallundae</i>	627 N	Ia	S	S	R	S
<i>Tapesia yallundae</i>	646 N	Ib	R	S	R	S
<i>Tapesia yallundae</i>	572 N	Ic	R	R	R	S
<i>Tapesia yallundae</i>	618 N	Ic	R	R	R	S
<i>Tapesia acuformis</i>	634 L	IIs	R	S	S	S
<i>Tapesia acuformis</i>	643 L	IIs	R	S	S	S
<i>Tapesia acuformis</i>	567 L	IIP	R	R	R	S
<i>Tapesia acuformis</i>	617 L	IIP	R	R	R	S
<i>Tapesia acuformis</i>	626 L	IIP	R	R	R	S

¹S = Sensitive,

R = Resistant

Example 2

Amplification of RAPD Products

Polymerase chain reactions are performed to obtain Randomly Amplified Polymorphic DNA (RAPD) profiles for each of the *Tapesia* spp. subtypes. Forty different RAPD 10-mer primers from Qiagen Operon (Operon Technologies Inc., Alameda, Calif., USA) kits AA and J identified individually as OPAA-01–OPAA-20 and OPJ-01–OPJ-20 are used in amplifications to find RAPD products specific to subtypes Ic and IIP. A single 10-mer RAPD primer is used in RAPD-PCR reactions. Reactions are prepared using the GeneAmp Kit from Perkin-Elmer (Foster City, Calif.; part no. N808-0009) using 50 mM KCl, 2.0 mM MgCl₂, 10 mM

transformed into One Shot chemically competent bacterial cells using the TOPO TA Cloning Kit for Sequencing (Invitrogen Corporation, Carlsbad, Calif., USA) under manufacturer's protocol. Transformed cells containing the vector plus RAPD-PCR product insert are identified by endonuclease digestion of miniprep DNA of isolated colonies. Minipreps of vector DNA containing the RAPD-PCR product are sequenced. Sequencing is performed on an ABI PRISM 377™ DNA sequencer (Applied Biosystems, Foster City, Calif.) using primers in the pCR4-TOPO cloning vector: FORWARD (5'-gtaaaacgacgccagct-3'; SEQ ID NO: 1) and REVERSE (5'-caggaacagctatgac-3'; SEQ ID NO: 2). Sequences obtained for each *Tapesia* spp. subtype are identified in Table 2.

TABLE 2

Tapesia spp. subtype-specific RAPD-PCR product sequences			
Sequence Identifier	Fungal Species	Subtype and Sequence ID	RAPD-PCR Sequence Length
SEQ-ID-NO: 3	<i>Tapesia yallundae</i>	Ib1-27	525
SEQ-ID-NO: 4	<i>Tapesia yallundae</i>	Ib2-31	551
SEQ-ID-NO: 5	<i>Tapesia yallundae</i>	Ib3-33	520
SEQ-ID-NO: 6	<i>Tapesia yallundae</i>	Ic1-22	456
SEQ-ID-NO: 7	<i>Tapesia yallundae</i>	Ic 020502Ic4and6	711
SEQ-ID-NO: 8	<i>Tapesia yallundae</i>	Ic 020602D-20	555
SEQ-ID-NO: 9	<i>Tapesia yallundae</i>	Ic 020602D-21	625
SEQ-ID-NO: 10	<i>Tapesia acuformis</i>	IIP1-17	455
SEQ-ID-NO: 11	<i>Tapesia acuformis</i>	IIP 020602A-11	498
SEQ-ID-NO: 12	<i>Tapesia acuformis</i>	IIP 020602B-15	702

TABLE 2-continued

Tapesia spp. subtype-specific RAPD-PCR product sequences			
Sequence Identifier	Fungal Species	Subtype and Sequence ID	RAPD-PCR Sequence Length
SEQ-ID-NO: 13	<i>Tapesia acuformis</i>	IIP 020602B-16	503
SEQ-ID-NO: 14	<i>Tapesia acuformis</i>	IIS2-39	513

Example 4

Design of Subtype-specific Primers

PCR Primers are designed to amplify within the sequences of RAPD-PCR products obtained according to Example 3. Primers are designed to be used either in conventional PCR reactions or with an oligonucleotide probe in TaqMan PCR reactions. Multiple primers are developed to target each RAPD-PCR *Tapesia* spp. subtype-specific sequence. These primers are listed in Table 3.

TABLE 3

Tapesia spp. subtype-specific primer sequences				
Sequence Identifier	Name	Species	Target DNA Subtype Prod-uct	RAPD-PCR Oligo Sequence (5'-3')
SEQ-ID-NO: 15	JB900	<i>Tapesia yallundae</i>	Ib	Ib1-27/Ib2-31 TGCGGTAGGGCGAAGAAAC
SEQ-ID-NO: 16	JB901	<i>Tapesia yallundae</i>	Ib	Ib1-27/Ib2-31 CATCTCCACCAACCAATACG
SEQ-ID-NO: 17	JB902 Probe	<i>Tapesia yallundae</i>	Ib	Ib1-27/Ib2-31 AACACCAAAGCGCTTCGCGAGA
SEQ-ID-NO: 18	JB903	<i>Tapesia yallundae</i>	Ib	Ib1-27/Ib2-31 CAGCCGATTGATCCGGTCTA
SEQ-ID-NO: 19	JB904	<i>Tapesia yallundae</i>	Ib	Ib1-27/Ib2-31 GGCAACGCTGATTCGACTCTA
SEQ-ID-NO: 20	JB905	<i>Tapesia yallundae</i>	Ib	Ib1-27/Ib2-31 GGTTCGATCCCGTCATCCT
SEQ-ID-NO: 21	JB906	<i>Tapesia yallundae</i>	Ib	Ib1-27/Ib2-31 GTCGGTTCGATCCCGTCAT
SEQ-ID-NO: 22	JB907	<i>Tapesia yallundae</i>	Ib	Ib1-27/Ib2-31 CAATACGCTCTGCGGTAGGGCGAA
SEQ-ID-NO: 23	JB908	<i>Tapesia yallundae</i>	Ib	Ib1-27/Ib2-31 GCCGCTTTGGTGTGGTTT
SEQ-ID-NO: 24	JB909	<i>Tapesia yallundae</i>	Ib	Ib1-27/Ib2-31 CAGCCGATTGATCCGGTCTAT
SEQ-ID-NO: 25	JB910	<i>Tapesia yallundae</i>	Ib	Ib3-33 AATTCGCCCTTGGACCTCTT
SEQ-ID-NO: 26	JB911	<i>Tapesia yallundae</i>	Ib	Ib3-33 TTCGCCCTTGGACCTCTTG
SEQ-ID-NO: 27	JB912 Probe	<i>Tapesia yallundae</i>	Ib	Ib3-33 AGTAACACGCCCCACGGACGGAT
SEQ-ID-NO: 28	JB913	<i>Tapesia yallundae</i>	Ib	Ib3-33 CTGCGGAGTCTCTGCTAGCT
SEQ-ID-NO: 29	JB914	<i>Tapesia yallundae</i>	Ib	Ib3-33 GACCTGCGGAGTCTCTGCT
SEQ-ID-NO: 30	JB915	<i>Tapesia yallundae</i>	Ic	Ic1-22 TTACTGTATTTGTCTGGTGATTGC
SEQ-ID-NO: 31	JB916	<i>Tapesia yallundae</i>	Ic	Ic1-22 GAGCCTCTCATATCTGGATCTCTAAATC
SEQ-ID-NO: 32	JB917 Probe	<i>Tapesia yallundae</i>	Ic	Ic1-22 TTAAC TAGCAGTCATCTGTCTGTGCCAAGG
SEQ-ID-NO: 33	JB918	<i>Tapesia yallundae</i>	Ic	Ic1-22 GACAACTCTACCAAGGAGAGACAAAA
SEQ-ID-NO: 34	JB919	<i>Tapesia yallundae</i>	Ic	Ic1-22 CTACCAAGGAGAGACAAAACAAAA
SEQ-ID-NO: 35	JB920	<i>Tapesia acuformis</i>	IIP	IIP1-17 TCTTGTGAGACTGCATGGACTAGAGT
SEQ-ID-NO: 36	JB921	<i>Tapesia acuformis</i>	IIP	IIP1-17 GATCTTGTGAGACTGCATGGACTAG

TABLE 3-continued

<u>Tapesia spp. subtype-specific primer sequences</u>					
Sequence			Target DNA	RAPD-PCR	
Identifier	Name	Species	Subtype Product	Oligo Sequence (5'-3')	
SEQ-ID-NO: 37	JB922 Probe	<i>Tapesia acuformis</i>	IIp	IIp1-17	CATGCGAGAATTAAAGAGCTATAGTTGCGTGC
SEQ-ID-NO: 38	JB923	<i>Tapesia acuformis</i>	IIp	IIp1-17	CGCAATCCTTTCTCGACTTCTAA
SEQ-ID-NO: 39	JB924	<i>Tapesia acuformis</i>	IIp	IIp1-17	GTTTCGCAATCCTTTCTCGACTT
SEQ-ID-NO: 40	JB925	<i>Tapesia acuformis</i>	IIIs	IIIs2-39	GCAGAATTCGCCCTTAAGTCG
SEQ-ID-NO: 41	JB926	<i>Tapesia acuformis</i>	IIIs	IIIs2-39	TCTGCAGAATTCGCCCTTAAG
SEQ-ID-NO: 42	JB927 Probe	<i>Tapesia acuformis</i>	IIIs	IIIs2-39	AAGGTAGCCGTATCGGAAGGTGCGG
SEQ-ID-NO: 43	JB928	<i>Tapesia acuformis</i>	IIIs	IIIs2-39	CCAGAACGGAGGTGATCCAG
SEQ-ID-NO: 44	JB929	<i>Tapesia acuformis</i>	IIIs	IIIs2-39	TTCCAGAACGGAGGTGATCC
SEQ-ID-NO: 45	JB930	<i>Tapesia yallundae</i>	Ic	Ic1-22	ATATTCTTGCTGAATTGGTC
SEQ-ID-NO: 46	JB931	<i>Tapesia yallundae</i>	Ic	Ic1-22	CAAATTTATTTTCATCCTTGGCACAG
SEQ-ID-NO: 47	JB932	<i>Tapesia yallundae</i>	Ic	Ic1-22	AAATTTATTTTCATCCTTGGCACAGG
SEQ-ID-NO: 48	JB933	<i>Tapesia yallundae</i>	Ic	Ic1-22	ATATTCTTGCTGAATTGGTC
SEQ-ID-NO: 49	JB934	<i>Tapesia acuformis</i>	IIp	IIp1-17	AGATGGGCAGAGTGTAGATCTTGTG
SEQ-ID-NO: 50	JB935	<i>Tapesia acuformis</i>	IIp	IIp1-17	GGAACCGAGAGTAGCAACAGAAC
SEQ-ID-NO: 51	JB936	<i>Tapesia acuformis</i>	IIp	IIp1-17	CAGGAACCGAGAGTAGCAACAG
SEQ-ID-NO: 52	JB937	<i>Tapesia acuformis</i>	IIp	IIp1-17	GCGTTCGGCTTGAAGTCATG
SEQ-ID-NO: 53	JB938	<i>Tapesia acuformis</i>	Ic	0205021c4and6	CCTTTGGTCGGGTGGGAGAA
SEQ-ID-NO: 54	JB939	<i>Tapesia acuformis</i>	Ic	0205021c4and6	GCCAGGCTGAATCTTGGGAA
SEQ-ID-NO: 55	JB940	<i>Tapesia acuformis</i>	Ic	0205021c4and6	CCAGGCTGAATCTTGGGAAA
SEQ-ID-NO: 56	JB941	<i>Tapesia acuformis</i>	Ic	0205021c4and6	CCAAGTACGCATCTCGGATG
SEQ-ID-NO: 57	JB942	<i>Tapesia acuformis</i>	Ic	020602D-20	GAAGTGTCTTACTCTTTGCCG
SEQ-ID-NO: 58	JB943	<i>Tapesia acuformis</i>	Ic	020602D-20	AATATTGGTTCTTGATCCTG
SEQ-ID-NO: 59	JB944	<i>Tapesia acuformis</i>	Ic	020602D-20	TCGAGACAATAGAGATTTTC
SEQ-ID-NO: 60	JB945	<i>Tapesia acuformis</i>	Ic	020602D-20	GTGTGTCATTTTGAAGATT
SEQ-ID-NO: 61	JB946	<i>Tapesia acuformis</i>	Ic	020602D-21	ACATACCATCTTGTAATAGCC
SEQ-ID-NO: 62	JB947	<i>Tapesia acuformis</i>	Ic	020602D-21	CATAGTCAATCCAAGCTTTC
SEQ-ID-NO: 63	JB948	<i>Tapesia acuformis</i>	Ic	020602D-21	ATACCATCTTGTAATAGCC
SEQ-ID-NO: 64	JB949	<i>Tapesia acuformis</i>	Ic	020602D-21	TATGCTTCTGGTCTTTGTTT
SEQ-ID-NO: 65	JB950	<i>Tapesia yallundae</i>	IIp	020602A-11	AATCAATGTCATCGGTTTCG
SEQ-ID-NO: 66	JB951	<i>Tapesia yallundae</i>	IIp	020602A-11	CACTTCCACGGCAGTGATAA
SEQ-ID-NO: 67	JB952	<i>Tapesia yallundae</i>	IIp	020602A-11	TTGTCTCTTGGGTAATCATG
SEQ-ID-NO: 68	JB953	<i>Tapesia yallundae</i>	IIp	020602A-11	GTGCCAAAAGGAAGTATTG
SEQ-ID-NO: 69	JB954	<i>Tapesia yallundae</i>	IIp	020602B-16	TGAGATTCGGACTGCATT
SEQ-ID-NO: 70	JB955	<i>Tapesia yallundae</i>	IIp	020602B-16	CAAAGTGAAGTTTCTCAACG
SEQ-ID-NO: 71	JB956	<i>Tapesia yallundae</i>	IIp	020602B-15	CCTTACCCGACCTGCCATGT
SEQ-ID-NO: 72	JB957	<i>Tapesia yallundae</i>	IIp	020602B-15	CTGGCGGCCATATCGACTTC

TABLE 3-continued

<u>Tapesia spp. subtype-specific primer sequences</u>					
Sequence			Target DNA	RAPD-PCR	
Identifier	Name	Species	Subtype Prod-uct	Oligo Sequence (5'-3')	
SEQ-ID-NO: 73	JB958	<i>Tapesia yallundae</i>	IIp	020602B-15	ATTAGCAACTGGAATGCACA
SEQ-ID-NO: 74	JB959	<i>Tapesia yallundae</i>	IIp	020602B-15	AAGCCAGCTGCATGATGTTC
SEQ-ID-NO: 75	JB960	<i>Tapesia yallundae</i>	IIp	020602B-16	CGCCCTAGCACATCATCAAA
SEQ-ID-NO: 76	JB961	<i>Tapesia yallundae</i>	IIp	020602B-16	CCTAGCACATCATCAAAAGA
SEQ-ID-NO: 77	JB962	<i>Tapesia yallundae</i>	IIp	020602B-16	GGAGCATGGAAGCACTCGTA

Example 5

Synthesis and Purification of Oligonucleotides

Oligonucleotides (primers) are synthesized by, for example, either Integrated DNA Technologies (Coralville, Iowa) or Midland Certified Reagent Company (Midland, Tex.). Primer sequences labeled as "probe" are synthesized with a fluorescent reporter group attached at the 5' end for example 6-carboxy-fluorescein or "FAM" and a fluorescence quenching group attached at the 3' end for example 6-carboxy-tetramethyl-rhodamine or "TAMRA" or for example a dark quencher such as the proprietary Black Hole Quencher or "BHQ™" from Biosearch Technologies (Novato, Calif.).

Example 6

Polymerase Chain Reaction Amplification

Polymerase chain reactions are performed with the GeneAmp Kit from Perkin-Elmer (Foster City, Calif.; part no. N808-0009) using 50 mM KCl, 2.5 mM MgCl₂, 10 mM

Tris-HCl, pH8.3, containing 200 μM of each dTTP, dATP, dCTP, and dGTP in 25 μL reactions containing 50 μM each primer, 0.25 U/μL of Taq polymerase and approximately 25 ng of genomic DNA per reaction. Reactions are run for 30–35 cycles of 15 s at 94° C., 15 s at 50° C.–70° C., and 45 s at 72° C. in a Perkin-Elmer Model 9600 or 9700 thermal cycler. The products are analyzed by loading 10 μl of each PCR sample on a 1.0% agarose gel and electrophoresing. The gel is stained with ethidium bromide and products are visualized under ultraviolet light.

Example 7

Determination of Primer Specificity to Purified Fungal Genomic DNA

PCRs are performed according to Example 6 using different primer combinations (Table 4) in an attempt to amplify single specific fragments. Specific PCR amplification products are produced from primers designed from RAPD-PCR product sequences of each *Tapesia* spp. subtype.

TABLE 4

Possible combinations of PCR primers for the specific amplification of <i>Tapesia</i> spp. subtypes Ic and IIp					
Target species subtype (RAPD-PCR Product)	Primer 1	Sequence Identifier	Primer 2	Sequence Identifier	
<i>Tapesia yallundae</i> Ib (Ib1-27/Ib2-31)	JB900	SEQ-ID-NO: 15	JB903	SEQ-ID-NO: 18	
<i>Tapesia yallundae</i> Ib (Ib1-27/Ib2-31)	JB900	SEQ-ID-NO: 15	JB904	SEQ-ID-NO: 19	
<i>Tapesia yallundae</i> Ib (Ib1-27/Ib2-31)	JB901	SEQ-ID-NO: 16	JB903	SEQ-ID-NO: 18	
<i>Tapesia yallundae</i> Ib (Ib1-27/Ib2-31)	JB901	SEQ-ID-NO: 16	JB904	SEQ-ID-NO: 19	
<i>Tapesia yallundae</i> Ib (Ib1-27/Ib2-31)	JB905	SEQ-ID-NO: 20	JB908	SEQ-ID-NO: 23	
<i>Tapesia yallundae</i> Ib (Ib1-27/Ib2-31)	JB905	SEQ-ID-NO: 20	JB909	SEQ-ID-NO: 24	
<i>Tapesia yallundae</i> Ib (Ib1-27/Ib2-31)	JB906	SEQ-ID-NO: 21	JB908	SEQ-ID-NO: 23	
<i>Tapesia yallundae</i> Ib (Ib1-27/Ib2-31)	JB906	SEQ-ID-NO: 21	JB909	SEQ-ID-NO: 24	
<i>Tapesia yallundae</i> Ib (Ib3-33)	JB910	SEQ-ID-NO: 25	JB913	SEQ-ID-NO: 28	
<i>Tapesia yallundae</i> Ib (Ib3-33)	JB910	SEQ-ID-NO: 25	JB914	SEQ-ID-NO: 29	
<i>Tapesia yallundae</i> Ib (Ib3-33)	JB911	SEQ-ID-NO: 26	JB913	SEQ-ID-NO: 28	

TABLE 4-continued

Possible combinations of PCR primers for the specific amplification of <i>Tapesia</i> spp. subtypes Ic and Ilp				
Target species subtype (RAPD-PCR Product)	Primer 1	Sequence Identifier	Primer 2	Sequence Identifier
<i>Tapesia yallundae</i> Ib (Ib3-33)	JB911	SEQ-ID-NO: 26	JB914	SEQ-ID-NO: 29
<i>Tapesia yallundae</i> Ic (Ic1-22)	JB915	SEQ-ID-NO: 30	JB918	SEQ-ID-NO: 33
<i>Tapesia yallundae</i> Ic (Ic1-22)	JB915	SEQ-ID-NO: 30	JB919	SEQ-ID-NO: 34
<i>Tapesia yallundae</i> Ic (Ic1-22)	JB916	SEQ-ID-NO: 31	JB918	SEQ-ID-NO: 33
<i>Tapesia yallundae</i> Ic (Ic1-22)	JB916	SEQ-ID-NO: 31	JB919	SEQ-ID-NO: 34
<i>Tapesia yallundae</i> Ic (Ic1-22)	JB920	SEQ-ID-NO: 35	JB923	SEQ-ID-NO: 38
<i>Tapesia yallundae</i> Ic (Ic1-22)	JB920	SEQ-ID-NO: 35	JB924	SEQ-ID-NO: 39
<i>Tapesia yallundae</i> Ic (Ic1-22)	JB921	SEQ-ID-NO: 36	JB923	SEQ-ID-NO: 38
<i>Tapesia yallundae</i> Ic (Ic1-22)	JB921	SEQ-ID-NO: 36	JB924	SEQ-ID-NO: 39
<i>Tapesia acufiformis</i> IIs (IIs2-39)	JB925	SEQ-ID-NO: 40	JB928	SEQ-ID-NO: 43
<i>Tapesia acufiformis</i> IIs (IIs2-39)	JB925	SEQ-ID-NO: 40	JB929	SEQ-ID-NO: 44
<i>Tapesia acufiformis</i> IIs (IIs2-39)	JB926	SEQ-ID-NO: 41	JB928	SEQ-ID-NO: 43
<i>Tapesia acufiformis</i> IIs (IIs2-39)	JB926	SEQ-ID-NO: 41	JB929	SEQ-ID-NO: 44
<i>Tapesia yallundae</i> Ic (Ic1-22)	JB930	SEQ-ID-NO: 45	JB931	SEQ-ID-NO: 46
<i>Tapesia yallundae</i> Ic (Ic1-22)	JB930	SEQ-ID-NO: 45	JB932	SEQ-ID-NO: 47
<i>Tapesia yallundae</i> Ic (Ic1-22)	JB933	SEQ-ID-NO: 48	JB931	SEQ-ID-NO: 46
<i>Tapesia yallundae</i> Ic (Ic1-22)	JB933	SEQ-ID-NO: 48	JB932	SEQ-ID-NO: 47
<i>Tapesia acufiformis</i> IIs/Ilp (Ilp1-17)	JB934	SEQ-ID-NO: 49	JB935	SEQ-ID-NO: 50
<i>Tapesia acufiformis</i> Ilp (Ilp1-17)	JB934	SEQ-ID-NO: 49	JB936	SEQ-ID-NO: 51
<i>Tapesia acufiformis</i> IIs/Ilp (Ilp1-17)	JB937	SEQ-ID-NO: 52	JB935	SEQ-ID-NO: 50
<i>Tapesia acufiformis</i> Ilp (Ilp1-17)	JB937	SEQ-ID-NO: 52	JB936	SEQ-ID-NO: 51
<i>Tapesia yallundae</i> Ic (0205021c4and6)	JB938	SEQ-ID-NO: 53	JB939	SEQ-ID-NO: 54
<i>Tapesia yallundae</i> Ic (0205021c4and6)	JB938	SEQ-ID-NO: 53	JB940	SEQ-ID-NO: 55
<i>Tapesia yallundae</i> Ic (0205021c4and6)	JB941	SEQ-ID-NO: 56	JB939	SEQ-ID-NO: 54
<i>Tapesia yallundae</i> Ic (0205021c4and6)	JB941	SEQ-ID-NO: 56	JB940	SEQ-ID-NO: 55
<i>Tapesia yallundae</i> Ic (020602D-20)	JB942	SEQ-ID-NO: 57	JB943	SEQ-ID-NO: 58
<i>Tapesia yallundae</i> Ic (020602D-20)	JB942	SEQ-ID-NO: 57	JB945	SEQ-ID-NO: 60
<i>Tapesia yallundae</i> Ic (020602D-20)	JB944	SEQ-ID-NO: 59	JB943	SEQ-ID-NO: 58
<i>Tapesia yallundae</i> Ic (020602D-20)	JB944	SEQ-ID-NO: 59	JB945	SEQ-ID-NO: 60
<i>Tapesia yallundae</i> Ic (020602D-21)	JB946	SEQ-ID-NO: 61	JB947	SEQ-ID-NO: 62
<i>Tapesia yallundae</i> Ic (020602D-21)	JB946	SEQ-ID-NO: 61	JB949	SEQ-ID-NO: 64
<i>Tapesia yallundae</i> Ic (020602D-21)	JB948	SEQ-ID-NO: 63	JB947	SEQ-ID-NO: 62
<i>Tapesia yallundae</i> Ic (020602D-21)	JB948	SEQ-ID-NO: 63	JB949	SEQ-ID-NO: 64
<i>Tapesia acufiformis</i> Ilp (020602A-11)	JB952	SEQ-ID-NO: 67	JB950	SEQ-ID-NO: 65
<i>Tapesia acufiformis</i> Ilp (020602A-11)	JB952	SEQ-ID-NO: 67	JB951	SEQ-ID-NO: 66
<i>Tapesia acufiformis</i> Ilp (020602A-11)	JB953	SEQ-ID-NO: 68	JB950	SEQ-ID-NO: 65
<i>Tapesia acufiformis</i> Ilp (020602A-11)	JB953	SEQ-ID-NO: 68	JB951	SEQ-ID-NO: 66
<i>Tapesia acufiformis</i> Ilp (020602B-16)	JB954	SEQ-ID-NO: 69	JB955	SEQ-ID-NO: 70
<i>Tapesia acufiformis</i> Ilp (020602B-16)	JB954	SEQ-ID-NO: 69	JB960	SEQ-ID-NO: 75
<i>Tapesia acufiformis</i> Ilp (020602B-16)	JB954	SEQ-ID-NO: 69	JB961	SEQ-ID-NO: 76
<i>Tapesia acufiformis</i> Ilp (020602B-16)	JB962	SEQ-ID-NO: 77	JB955	SEQ-ID-NO: 70
<i>Tapesia acufiformis</i> Ilp (020602B-16)	JB962	SEQ-ID-NO: 77	JB960	SEQ-ID-NO: 75
<i>Tapesia acufiformis</i> Ilp (020602B-16)	JB962	SEQ-ID-NO: 77	JB961	SEQ-ID-NO: 76
<i>Tapesia acufiformis</i> Ilp (020602B-15)	JB956	SEQ-ID-NO: 71	JB957	SEQ-ID-NO: 72
<i>Tapesia acufiformis</i> Ilp (020602B-15)	JB956	SEQ-ID-NO: 71	JB959	SEQ-ID-NO: 74
<i>Tapesia acufiformis</i> Ilp (020602B-15)	JB958	SEQ-ID-NO: 73	JB957	SEQ-ID-NO: 72
<i>Tapesia acufiformis</i> Ilp (020602B-15)	JB958	SEQ-ID-NO: 73	JB959	SEQ-ID-NO: 74

In an initial screen for specificity, PCR reaction mixtures are made according to Example 6 for each of the primer combinations in Table 4. These are run against a negative control (no DNA added) and approximately 25 ng of fungal DNA for each of the *Tapesia* spp. subtypes listed in Table 1 prepared as described in example 1.

When visualized on an ethidium bromide stained gel several primer pairs give satisfactory results: good amplifi-

cation of target DNA from multiple isolates of the target species subtype with all other reactions (negative control and other fungal DNAs) free of both specific and nonspecific reaction products. Some give unsatisfactory results including nonspecific amplification, no amplification of target DNA, and amplification of DNAs from fungal species other than the target. The primer pairs that give good specific amplification for *T. yallundae* subtype Ic target DNA with no cross-amplification are summarized in Table 5.

TABLE 5

PCR primer pairs providing specific and sensitive amplification of target DNAs for <i>Tapesia yallundae</i> subtype Ic.				
Target species subtype (RAPD-PCR Product)	Primer 1	Sequence Identifier	Primer 2	Sequence Identifier
<i>Tapesia yallundae</i> Ic (020602D-20)	JB944	SEQ-ID-NO: 59	JB943	SEQ-ID-NO: 58
<i>Tapesia yallundae</i> Ic (020602D-20)	JB944	SEQ-ID-NO: 59	JB945	SEQ-ID-NO: 60

When primers JB944 and JB943 or primers JB944 and JB945 are run against DNA preparations of the eyespot subtypes listed in Table 1 the following results are recorded (Table 6).

TABLE 6

Results of primer pairs JB944 and JB943 and JB944 and JB945 on test isolates of <i>Tapesia</i> spp.				
Fungal	Fungal	Subtype	PCR Results (+/-)	
Species	Isolate	Identifier	JB944/JB943	JB944/JB945
<i>Tapesia yallundae</i>	627 N	Ia	-	-
<i>Tapesia yallundae</i>	646 N	Ib	-	-
<i>Tapesia yallundae</i>	572 N	Ic	+	+
<i>Tapesia yallundae</i>	618 N	Ic	+	+
<i>Tapesia acuformis</i>	634 L	Iis	-	-
<i>Tapesia acuformis</i>	643 L	Iis	-	-
<i>Tapesia acuformis</i>	567 L	Iip	-	-
<i>Tapesia acuformis</i>	617 L	Iip	-	-
<i>Tapesia acuformis</i>	626 L	Iip	-	-

Thus, primer pairs JB944 and JB943 and primers JB944 and JB945 are useful in the differentiation of subtypes within the *Tapesia yallundae* species.

For specificity to *T. acuformis* subtypes IIs and Iip primer pairs were selected that amplify from both *T. acuformis* subtypes but with different sized PCR products that allow differentiation of the subtype by product size. Again, primers were selected based on good amplification of target DNA from multiple isolates of the target species subtype with all other reactions (negative control and other fungal DNAs) free of both specific and nonspecific reaction products. Some give unsatisfactory results including nonspecific amplification, no amplification of target DNA, and amplification of DNAs from fungal species other than the target. The primer pairs that give good specific amplification for *T. acuformis* subtypes IIs and Iip with different sized products for each subtype with no cross-amplification are summarized in Table 7.

TABLE 7

PCR primer pairs providing specific and sensitive amplification of target DNAs for <i>Tapesia acuformis</i> subtypes IIs and Iip.				
Target species subtype (RAPD-PCR Product)	Primer 1	Sequence Identifier	Primer 2	Sequence Identifier
<i>Tapesia acuformis</i> IIs/Iip (Iip1-17)	JB934	SEQ-ID-NO: 49	JB935	SEQ-ID-NO: 50
<i>Tapesia acuformis</i> IIs/Iip (Iip1-17)	JB937	SEQ-ID-NO: 52	JB935	SEQ-ID-NO: 50

Primers JB934 and JB935 are run against DNA preparations of the eyespot subtypes listed in Table 1. The results are presented in Table 8).

TABLE 8

Results of primer pairs JB934 and JB935 on test isolates of <i>Tapesia</i> spp.				
Fungal	Fungal	Subtype	PCR Results (+/-)	
Species	Isolate	Identifier	(+/-)	Amplification Product Size
<i>Tapesia yallundae</i>	627 N	Ia	-	-
<i>Tapesia yallundae</i>	646 N	Ib	-	-
<i>Tapesia yallundae</i>	572 N	Ic	-	-
<i>Tapesia yallundae</i>	618 N	Ic	-	-
<i>Tapesia acuformis</i>	634 L	IIs	+	~600
<i>Tapesia acuformis</i>	643 L	IIs	+	~600
<i>Tapesia acuformis</i>	567 L	Iip	+	~400
<i>Tapesia acuformis</i>	617 L	Iip	+	~400
<i>Tapesia acuformis</i>	626 L	Iip	+	~400

Thus, primer pairs JB934 and JB935 are specific to *Tapesia acuformis* at the species level and provide differently sized PCR products that are useful in the differentiation of IIs and Iip subtypes within the *Tapesia acuformis* species.

Example 8

TaqMan Based Detection of *Tapesia* spp. Subtypes

Some of the primers detailed in Table in 3 were designed for the additional possible use in TaqMan reactions for detection of specific *Tapesia* spp. subtypes. Possible primer combinations for these reactions are listed in Table 9.

TABLE 9

Possible combinations of TaqMan primers and probes for the specific amplification of <i>Tapesia</i> spp. subtypes						
Target species subtype (RAPD-PCR Product)	Primer 1	Sequence Identifier	Probe	Sequence Identifier	Primer 2	Sequence Identifier
<i>Tapesia yellundae</i> Ib (Ib1-27/Ib2-31)	JB900	SEQ-ID-NO: 15	JB902	SEQ-ID-NO: 17	JB903	SEQ-ID-NO: 18
<i>Tapesia yellundae</i> Ib (Ib1-27/Ib2-31)	JB900	SEQ-ID-NO: 15	JB902	SEQ-ID-NO: 17	JB904	SEQ-ID-NO: 19
<i>Tapesia yellundae</i> Ib (Ib1-27/Ib2-31)	JB901	SEQ-ID-NO: 16	JB902	SEQ-ID-NO: 17	JB903	SEQ-ID-NO: 18
<i>Tapesia yellundae</i> Ib (Ib1-27/Ib2-31)	JB901	SEQ-ID-NO: 16	JB902	SEQ-ID-NO: 17	JB904	SEQ-ID-NO: 19
<i>Tapesia yellundae</i> Ib (Ib1-27/Ib2-31)	JB905	SEQ-ID-NO: 20	JB907	SEQ-ID-NO: 22	JB908	SEQ-ID-NO: 23
<i>Tapesia yellundae</i> Ib (Ib1-27/Ib2-31)	JB905	SEQ-ID-NO: 20	JB907	SEQ-ID-NO: 22	JB909	SEQ-ID-NO: 24
<i>Tapesia yellundae</i> Ib (Ib1-27/Ib2-31)	JB906	SEQ-ID-NO: 21	JB907	SEQ-ID-NO: 22	JB908	SEQ-ID-NO: 23
<i>Tapesia yellundae</i> Ib (Ib1-27/Ib2-31)	JB906	SEQ-ID-NO: 21	JB907	SEQ-ID-NO: 22	JB909	SEQ-ID-NO: 24
<i>Tapesia yellundae</i> Ib (Ib3-33)	JB910	SEQ-ID-NO: 25	JB912	SEQ-ID-NO: 27	JB913	SEQ-ID-NO: 28
<i>Tapesia yellundae</i> Ib (Ib3-33)	JB910	SEQ-ID-NO: 25	JB912	SEQ-ID-NO: 27	JB914	SEQ-ID-NO: 29
<i>Tapesia yellundae</i> Ib (Ib3-33)	JB911	SEQ-ID-NO: 26	JB912	SEQ-ID-NO: 27	JB913	SEQ-ID-NO: 28
<i>Tapesia yellundae</i> Ib (Ib3-33)	JB911	SEQ-ID-NO: 26	JB912	SEQ-ID-NO: 27	JB914	SEQ-ID-NO: 29
<i>Tapesia yellundae</i> Ic (Ic1-22)	JB915	SEQ-ID-NO: 30	JB917	SEQ-ID-NO: 32	JB918	SEQ-ID-NO: 33
<i>Tapesia yellundae</i> Ic (Ic1-22)	JB915	SEQ-ID-NO: 30	JB917	SEQ-ID-NO: 32	JB919	SEQ-ID-NO: 34
<i>Tapesia yellundae</i> Ic (Ic1-22)	JB916	SEQ-ID-NO: 31	JB917	SEQ-ID-NO: 32	JB918	SEQ-ID-NO: 33
<i>Tapesia yellundae</i> Ic (Ic1-22)	JB916	SEQ-ID-NO: 31	JB917	SEQ-ID-NO: 32	JB919	SEQ-ID-NO: 34
<i>Tapesia yellundae</i> Ic (Ic1-22)	JB920	SEQ-ID-NO: 35	JB922	SEQ-ID-NO: 37	JB923	SEQ-ID-NO: 38
<i>Tapesia yellundae</i> Ic (Ic1-22)	JB920	SEQ-ID-NO: 35	JB922	SEQ-ID-NO: 37	JB924	SEQ-ID-NO: 39
<i>Tapesia yellundae</i> Ic (Ic1-22)	JB921	SEQ-ID-NO: 36	JB922	SEQ-ID-NO: 37	JB923	SEQ-ID-NO: 38
<i>Tapesia yellundae</i> Ic (Ic1-22)	JB921	SEQ-ID-NO: 36	JB922	SEQ-ID-NO: 37	JB924	SEQ-ID-NO: 39
<i>Tapesia acufornis</i> IIs (IIs2-39)	JB925	SEQ-ID-NO: 40	JB927	SEQ-ID-NO: 42	JB928	SEQ-ID-NO: 43
<i>Tapesia acufornis</i> IIs (IIs2-39)	JB925	SEQ-ID-NO: 40	JB927	SEQ-ID-NO: 42	JB929	SEQ-ID-NO: 44
<i>Tapesia acufornis</i> IIs (IIs2-39)	JB926	SEQ-ID-NO: 41	JB927	SEQ-ID-NO: 42	JB928	SEQ-ID-NO: 43
<i>Tapesia acufornis</i> IIs (IIs2-39)	JB926	SEQ-ID-NO: 41	JB927	SEQ-ID-NO: 42	JB929	SEQ-ID-NO: 44

The combinations listed in Table 7 are tested in initial TaqMan™ screens for subtype level specificity. Primer and probe combinations are tested for their ability to amplify from the target subtypes's DNA. Reaction conditions are held constant (1× TaqMan™ Universal Master Mix (Perkin Elmer, Norwalk, Conn.; part no. N430-4447), 300 nM each primer, 200 nM probe, approximately 25 ng pre reaction of fungal target genomic DNA, thermal cycling: 50° C. for 2 min., 95° C. for 10 min., 40 cycles of 95° C. for 15 s, 60° C. for 60 s). In initial screens for specificity under these conditions no primer/probe combination provides absolute specificity. It is prophetic that further experimentation with reaction conditions will provide subtype specific tests using these primers that are designed for specificity.

This invention also provides the possibility of assessing potential damage in a specific crop variety-pathogen strain relationship and of utilizing judiciously the diverse armory

of fungicides which is available. Furthermore, it can be used to provide detailed information on the development and spread of specific pathogen races over extended geographical areas.

Kits useful in the practice of the invention are also provided. The kits find particular use in the identification of *Tapesia* pathogens.

While the present invention has been described with reference to specific embodiments thereof, it will be appreciated that numerous variations, modifications, and further embodiments are possible, and accordingly, all such variations, modifications and embodiments are to be regarded as being within the scope of the present invention.

Numerous patents, applications and references are discussed or cited within this specification, and all are incorporated by reference in their entireties.

SEQUENCE LISTING

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<223> OTHER INFORMATION: primer
<220> FEATURE:
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<400> SEQUENCE: 2

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17

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 aaacagcatt caatattgaa cgccacact tatcggttgt tggagaagt cgtgctaacc 180
 cgacatgggt ctgtagctca gctggttaga gcaccgtctt gataaggcgg ggtcgttg 240
 ttcgagccca actagaccca ccaaattctc cgaacataag atcgaggat cagtggggga 300
 ttagctcagc tgggagagca cctgctttgc aagcaggggg tcgtcggttc gatcccgta 360
 tcctccacca accaatacgc tctgcgtag ggcgaagaaa ccaacaccaa agcggcttcg 420
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 aacagcattc aatattgaac gccacacatt atcggttgtt ggaagaagtc ggtgctaacc 180
 gacatgggtc tgtagctcag ctggttagag caccgtcttg ataaggcggg ggtcgttggt 240
 tcgagcccaa ctagaccac caaatcttcc gaacataaga tgcgaggatc agtgggggat 300
 tagctcagct gggagagcac ctgctttgca agcagggggg cgtcggttcg atcccgta 360
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 gagaggcctc tttgttgttg gtccggtata gaccggatca atcggctgtt ctttaaaaat 480
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 <213> ORGANISM: Tapesia yallundae

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ctactttag tagccctgg tatggactcg gcattttaca aagtaaattc tcatgaaata	180
tgttttgggc tcggctaattg attgtagggc tttggtctca acattagtaa cgagctggta	240
aaagtgaagg cgcaatgct acctgcaccg accctgttgt tcaagggcaa taagccagat	300
aagggtcagg atagcttggg actggggtag aagggaacgt ggaacctatc agatatcaca	360
ttttactcgc ccggtaggcc atctgacgaa accgaatacc tcgaactagg gttcatcaag	420
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tggttttttc tcttgccatt tttaggattc actcttcact ttgacttcag acagtctgat	180
tataatgttg ccatgggtga gaccttttta cactgtattt gtctggtgat tgctgagcct	240
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ataattttgt gttttgtctc tccttggtag agtttgtcac cccaagtaa gaactaaggc	360
tctgagaaaa taagttttgc ccaacctaa tgtagtatgt tcaagtcaga tatttaaacc	420
taatccaaca tttcttgccc caaccatctc tataag	456

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 <213> ORGANISM: Tapesia yallundae

<400> SEQUENCE: 7

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cctcgctgtc ctcggaatcc atgccttcgt ctattgtact ctccgggggc gtttccctt	180
ccgcatatcg aggggagggt tgtgggttct tcagctgtgt aacctgggt gatccatctc	240
ccatttgca atcgctgaag ctctttcgat gttcgaggtc cttgagcgta agttttggcc	300
catcattgtc ggattggact tcagaggcca cactttcctc cagggttct tccacctcg	360
tgtocccatt ggcgctgggc gatgcatcga ccgtgaaaga actagtattt ccccgcttct	420
tctcgagttt cccaagattc agcctggcct ctttcattgt tattcgtgtc ttgaccttt	480
cgaacactgt cccaaccatc tgtgtcaaag tcccctgcgc aacctgctgg ttcgactgc	540
ttttcgaaag aagggaacaca ttgtagacct gcctgactgc cttcaatagt ccagcgccgt	600
gtaccacaat tttgtcatc agcacagctg cgagtaacga ctttacaatc tgtaactgga	660
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gctccaacag cagaggggct ttcttttgat ctgcctgaag atggacagcc tcatgttggg	180
ccgaattcat cgtcagacta cacagccttg ctgcccgaatt gacgcccggc tgctgagcat	240
cacgtgttac cacttttcca gctcagctca gcttagtctg atcttcttcc atttcaagtc	300
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ggggtagaat agaca	555

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 <212> TYPE: DNA
 <213> ORGANISM: *Tapesia yallundae*

<400> SEQUENCE: 9

caaggaatg atacacgtga ctgggtctgg atgagtccaa aaatagaaga acataccatc	60
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 <212> TYPE: DNA
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gctatagtgt cgtgcaatta gaagtcgaga aaggattgag aaacttcaat atgacaatcg	180
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aaaaggaact gattgactgt gattgtcaac aacctatat tcaggaatat ttccctaactt    180
gaagctgccc attctctccg tccgtgcgta gtcattcatc ctccgatcct caaaacttca    240
ccaccgattc atcaacccat gtttaatcat aaatttctgg agctgtcaag ctccactttg    300
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<212> TYPE: DNA

<213> ORGANISM: *Tapesia acuformis*

<400> SEQUENCE: 12

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caactggaat gcacaactcg agattgcaat caagctctct ctcttaccct caattaccaa    180
ccatgttttc accgagataa gagtgtcctc cgggtccaga atgaatccca gtccattcaa    240
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ctcttgcttc tcgattccgc ctccctgttg ctggctccct caggtcgggt aagggcgaat    660
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<210> SEQ ID NO 13

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<212> TYPE: DNA

<213> ORGANISM: *Tapesia acuformis*

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gaggacaga gatattcaga ggagcatgga agcactcgta aagctcagga tcatatgctg    180
gcggggtaaa ataggcttg cagggcggag cgaggggagc tgagggtccg ccagcaagaa    240
gtccttaagc aatgtctata agtacgtta tactatttgt cgcacctaag gactatcata    300
actogaataa agtaaagtaa agtcctgcat cgtttcaaga ctttgatatc atttcatgag    360
ttgagaaatc tcagtttgcc catcttttga tgatgtgcta gggcggagct ccaactccact    420
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aaactgcatt caatattgaa cgcccacact tatcggttgt tggaagagtc ggtctgaccg      180
acatgggtct gtagctcagc tggtttagagc accgtcttga taaggcgggg gtcgttggtt      240
cgagcccaac tagaccaccc aaatcttcca aacatcagat gcgaggatca ttgggggatt      300
agctcagctg ggagagcacc tgctttgcaa gcagggggtc gtcggttcga tcccgtcac      360
ctccaccaac caatcgggat caatgcaaca ccaaagaggc tttgaaaaag gcttctttgt      420
tgttgatcga gattactcag atcaatcggc tgttctttaa aaattcatag agtcgaatca      480
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20

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<223> OTHER INFORMATION: primer
<220> FEATURE:
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<222> LOCATION: (1)..(21)

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21

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<223> OTHER INFORMATION: primer
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<222> LOCATION: (1)..(19)

<400> SEQUENCE: 20

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19

<210> SEQ ID NO 21
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<220> FEATURE:
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<222> LOCATION: (1)..(19)

<400> SEQUENCE: 21

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19

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24

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<223> OTHER INFORMATION: primer
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<400> SEQUENCE: 23

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gccgctttgg tggtggttt 19

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<221> NAME/KEY: misc_feature
<222> LOCATION: (1)..(21)

<400> SEQUENCE: 24

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<400> SEQUENCE: 25

aattcgccct tggacctctt 20

<210> SEQ ID NO 26
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<212> TYPE: DNA
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<400> SEQUENCE: 26

ttcgcccttg gacctcttg 19

<210> SEQ ID NO 27
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<212> TYPE: DNA
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<220> FEATURE:
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<400> SEQUENCE: 27

agtaacacgc cccacggacg gat 23

<210> SEQ ID NO 28
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<212> TYPE: DNA
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<400> SEQUENCE: 28

ctgcggagtc cttgctagct 20

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<210> SEQ ID NO 29
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<400> SEQUENCE: 29

gacctgcgga gtccttgct

19

<210> SEQ ID NO 30
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<220> FEATURE:
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<400> SEQUENCE: 30

ttacactgta ttgtctggt gattgc

26

<210> SEQ ID NO 31
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<212> TYPE: DNA
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<220> FEATURE:
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<400> SEQUENCE: 31

gagcctctca tatctggatc tctaaatc

28

<210> SEQ ID NO 32
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<220> FEATURE:
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<220> FEATURE:
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<222> LOCATION: (1)..(31)

<400> SEQUENCE: 32

ttaactagca gtcactgtc ctgtgccaaag g

31

<210> SEQ ID NO 33
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<212> TYPE: DNA
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<220> FEATURE:
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<220> FEATURE:
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<222> LOCATION: (1)..(27)

<400> SEQUENCE: 33

gacaaactct accaaggaga gacaaaa

27

<210> SEQ ID NO 34
<211> LENGTH: 26
<212> TYPE: DNA
<213> ORGANISM: artificial sequence

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<220> FEATURE:
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<220> FEATURE:
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<400> SEQUENCE: 34

ctaccaagga gagacaaaac acaaaa

26

<210> SEQ ID NO 35
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<220> FEATURE:
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<400> SEQUENCE: 35

tcttgtgaga ctgcatggac tagagt

26

<210> SEQ ID NO 36
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<220> FEATURE:
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<400> SEQUENCE: 36

gatcttgtga gactgcatgg actag

25

<210> SEQ ID NO 37
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<220> FEATURE:
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<222> LOCATION: (1)..(32)

<400> SEQUENCE: 37

catgcgagaa ttaaagagct atagttgcgt gc

32

<210> SEQ ID NO 38
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<400> SEQUENCE: 38

cgcaatcctt tctcgacttc taa

23

<210> SEQ ID NO 39
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<222> LOCATION: (1)..(23)

<400> SEQUENCE: 39

gtttcgcaat cctttctcga ctt

23

<210> SEQ ID NO 40

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<212> TYPE: DNA

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<220> FEATURE:

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<220> FEATURE:

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<222> LOCATION: (1)..(21)

<400> SEQUENCE: 40

gcagaattcg cccttaagtc g

21

<210> SEQ ID NO 41

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<220> FEATURE:

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<400> SEQUENCE: 41

tctgcagaat tcgcccttaa g

21

<210> SEQ ID NO 42

<211> LENGTH: 25

<212> TYPE: DNA

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<220> FEATURE:

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<220> FEATURE:

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<222> LOCATION: (1)..(25)

<400> SEQUENCE: 42

aaggtagccg tatcggaagg tgcgg

25

<210> SEQ ID NO 43

<211> LENGTH: 20

<212> TYPE: DNA

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<220> FEATURE:

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<220> FEATURE:

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<222> LOCATION: (1)..(20)

<400> SEQUENCE: 43

ccagaacgga ggtgatccag

20

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<212> TYPE: DNA

<213> ORGANISM: artificial sequence

<220> FEATURE:

<223> OTHER INFORMATION: primer

<220> FEATURE:

<221> NAME/KEY: misc_feature

<222> LOCATION: (1)..(20)

<400> SEQUENCE: 44

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ttccagaacg gaggtgatcc 20

<210> SEQ ID NO 45
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<220> FEATURE:
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<222> LOCATION: (1)..(20)

<400> SEQUENCE: 45

atattcttgc tgaattggtc 20

<210> SEQ ID NO 46
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<212> TYPE: DNA
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: primer
<220> FEATURE:
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<222> LOCATION: (1)..(25)

<400> SEQUENCE: 46

caaaattatt tcattccttgg cacag 25

<210> SEQ ID NO 47
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<212> TYPE: DNA
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: primer
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (1)..(24)

<400> SEQUENCE: 47

aaattatttc atccttgga cagg 24

<210> SEQ ID NO 48
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<212> TYPE: DNA
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<220> FEATURE:
<223> OTHER INFORMATION: primer
<220> FEATURE:
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<222> LOCATION: (1)..(20)

<400> SEQUENCE: 48

atattcttgc tgaattggtc 20

<210> SEQ ID NO 49
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<212> TYPE: DNA
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<220> FEATURE:
<223> OTHER INFORMATION: primer
<220> FEATURE:
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<222> LOCATION: (1)..(25)

<400> SEQUENCE: 49

agatgggcag agttagatc ttgtg 25

<210> SEQ ID NO 50

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<211> LENGTH: 25
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<213> ORGANISM: artificial sequence
<220> FEATURE:
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<222> LOCATION: (1)..(25)

<400> SEQUENCE: 50

ggaaccgaga gagtagcaac agaac

25

<210> SEQ ID NO 51
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<212> TYPE: DNA
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: primer
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (1)..(24)

<400> SEQUENCE: 51

caggaaccga gagagtagca acag

24

<210> SEQ ID NO 52
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<220> FEATURE:
<223> OTHER INFORMATION: primer
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<222> LOCATION: (1)..(20)

<400> SEQUENCE: 52

gcgttcggct tgaagtcag

20

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<223> OTHER INFORMATION: primer
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<400> SEQUENCE: 53

cctttggtcg ggtgggagaa

20

<210> SEQ ID NO 54
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<222> LOCATION: (1)..(20)

<400> SEQUENCE: 54

gccaggtga atcttgggaa

20

<210> SEQ ID NO 55
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<220> FEATURE:

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<223> OTHER INFORMATION: primer
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (1)..(20)

<400> SEQUENCE: 55

ccaggctgaa tcttgggaaa

20

<210> SEQ ID NO 56
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<222> LOCATION: (1)..(20)

<400> SEQUENCE: 56

ccaagtacgc atctcggatg

20

<210> SEQ ID NO 57
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<212> TYPE: DNA
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<400> SEQUENCE: 57

gaagtgttta ctctttgccg

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<210> SEQ ID NO 58
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<222> LOCATION: (1)..(20)

<400> SEQUENCE: 58

aatattgggtt cttgatcctg

20

<210> SEQ ID NO 59
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<212> TYPE: DNA
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<220> FEATURE:
<223> OTHER INFORMATION: primer
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<222> LOCATION: (1)..(20)

<400> SEQUENCE: 59

tcgagacaat agagattttc

20

<210> SEQ ID NO 60
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<212> TYPE: DNA
<213> ORGANISM: artificial sequence
<220> FEATURE:
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<220> FEATURE:
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<400> SEQUENCE: 60

tcgagacaat agagattttc

20

<210> SEQ ID NO 61

<211> LENGTH: 22

<212> TYPE: DNA

<213> ORGANISM: artificial sequence

<220> FEATURE:

<223> OTHER INFORMATION: primer

<220> FEATURE:

<221> NAME/KEY: misc_feature

<222> LOCATION: (1)..(22)

<400> SEQUENCE: 61

acataccatc ttgtaaataag cc

22

<210> SEQ ID NO 62

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<212> TYPE: DNA

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<222> LOCATION: (1)..(20)

<400> SEQUENCE: 63

ataccatctt gtaaataagcc

20

<210> SEQ ID NO 64

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<212> TYPE: DNA

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<221> NAME/KEY: misc_feature

<222> LOCATION: (1)..(20)

<400> SEQUENCE: 64

tatgcttctg gtctttgttt

20

<210> SEQ ID NO 65

<211> LENGTH: 20

<212> TYPE: DNA

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<222> LOCATION: (1)..(20)

<400> SEQUENCE: 65

aatcaatgtc atgcggttcg

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<210> SEQ ID NO 66
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<220> FEATURE:
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<222> LOCATION: (1)..(20)

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cacttccacg gcagtgataa

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<210> SEQ ID NO 67
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<222> LOCATION: (1)..(20)

<400> SEQUENCE: 67

ttgtctcttg ggtaatcatg

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<210> SEQ ID NO 68
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gtgccaaaag gaactgattg

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<210> SEQ ID NO 69
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<400> SEQUENCE: 69

tgagattccg gactgcattt

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<210> SEQ ID NO 70
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<400> SEQUENCE: 70

caaactgaga tttctcaacg

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<210> SEQ ID NO 71
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<212> TYPE: DNA
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<400> SEQUENCE: 71

ccttaccgga cctgccatgt

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<400> SEQUENCE: 72

ctggcggcca tatcgacttc

20

<210> SEQ ID NO 73
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<212> TYPE: DNA
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<222> LOCATION: (1)..(20)

<400> SEQUENCE: 73

attagcaact ggaatgcaca

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<210> SEQ ID NO 74
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<212> TYPE: DNA
<213> ORGANISM: artificial sequence
<220> FEATURE:
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<220> FEATURE:
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<222> LOCATION: (1)..(20)

<400> SEQUENCE: 74

aagccagctg catgatgttc

20

<210> SEQ ID NO 75
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<212> TYPE: DNA
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<220> FEATURE:
<223> OTHER INFORMATION: primer
<220> FEATURE:
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<222> LOCATION: (1)..(20)

<400> SEQUENCE: 75

cgccctagca catcatcaaa

20

<210> SEQ ID NO 76
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<212> TYPE: DNA
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<220> FEATURE:
<223> OTHER INFORMATION: primer

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<222> LOCATION: (1)..(20)
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20

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ggagcatgga agcactcgta
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20

What is claimed is:

1. An isolated nucleic acid molecule comprising the nucleic acid sequence of SEQ ID NO: 8.

2. A pair of oligonucleotide primers wherein at least one primer consists of the nucleotide sequence of SEQ ID NO: 58 or SEQ ID NO: 59.

3. A pair of oligonucleotide primers comprising: JB944 (SEQ ID NO: 59) and JB943 (SEQ ID NO: 58).

4. A method for the detection of a fungal pathogen, comprising the steps of:

(a) isolating DNA from a plant tissue infected with a pathogen;

(b) subjecting said DNA to polymerase chain reaction amplification using at least one primer that comprises the sequence of SEQ ID NO: 58 or SEQ ID NO: 59 and

(c) detecting said fungal pathogen by visualizing the product or products of said polymerase chain reaction amplification.

5. The method of claim 4, wherein the fungal pathogen is *Tapesia yallundae*, *Tapesia acuformis*.

6. The method of claim 5, wherein the *Tapesia yallundae* is subtype Ic., *Tapesia acuformis* subtypes IIa or IIp.

7. A diagnostic kit used in detecting a fungal pathogen having at least one primer that comprises SEQ ID NO: 58 or SEQ ID NO: 59.

8. The diagnostic kit of claim 7, wherein the pair of primers are JB944 (SEQ ID NO: 59) and JB943 (SEQ ID NO: 58).

* * * * *